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Controversies and Counterpoints

Bone Matrix Proteins: More Than Markers

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At the end of many meetings on calcified tissue, I often walk away with the impression that mineralized matrix is largely considered a massive, inert "support" for bone cells. In other words, the role of the mineralized matrix is merely regarded as one of storage, serving as a reservoir for more important things like growth factors. One might even admit that the matrix provides a source of markers to monitor the activities and health status of bone tissue. The goal of this editorial is to convince the reader that matrix proteins themselves have critical roles in calcified tissue at many levels including regulation of cell differentiation, growth factor activity, and tissue integrity and function.

Matrix Proteins as Bone Barometer

Biochemical analysis of skeletal tissues has shown that, on a per weight basis, the matrix proteins are predominant, occurring in micromolar concentrations. By comparison, most growth factors within the same matrices exist in the nanomolar range and have a much shorter half-life. During normal aging there is a tightly controlled balance of bone formation and resorption. When these two events are not synchronized, normal bone mass is reduced, leading to osteoporosis. Biochemists have capitalized on the fact that during this synthesis and degradation, both intact and degraded matrix proteins are released into body fluids and subsequently can be used to monitor the relative rate of the two opposing processes. Indeed 9 of the 11 bone markers commonly used to measure bone formation or resorption are matrix proteins. They include osteocalcin (OC) and many variants derived from collagen production and modification [1]. The two remaining markers, tartrate-resistant acid phosphatase and alkaline phosphatase, are not typically considered matrix proteins but are likely to play roles in matrix modification and bone tissue function. Undoubtedly, additional biochemical analysis of mineralized tissues will reveal even more markers that will be developed into useful assays for bone disease (i.e., BSP). Moreover, they could be used to monitor other diseases important to the skeleton including osteogenesis imperfecta (OI), osteoarthritis (OA), fibrous dysplasia (FD), or metastatic tumor formation.

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"Give Me Shelter"

An overriding paradigm for bone matrix is that it serves simply as a scaffold, or a framework for bone tissue. It provides support and strength that are key elements in bone tissue function. Evidence that the matrix composition plays an important role in the skeleton comes from numerous reports of genetic defects in collagen, resulting in "brittle bone" or osteogenesis imperfecta [2, 3]. Furthermore, several recent reports showed that there are genetic polymorphisms in the regulatory regions of the type I collagen genes that can be linked to osteoporosis and fracture incidence [4]. Corresponding mRNA and protein analysis indicate that these relationships are likely based upon a chemical imbalance of COLIA1 to COLIA2 chains in the collagen triple helix [5]. Interestingly, other matrix proteins, including osteonectin and the small proteoglycans, may directly bind collagen and regulate its triple helical structure and function [6]. With one matrix protein affecting another, one can anticipate a complicated "domino" effect resulting from changes in the level of a single matrix component. Considering the fact that growth factors are powerful inducers of matrix production, it is possible that they also affect bone by regulating proper balance of individual matrix components. Moreover, in addition to a structural role, matrix proteins could affect each other, establishing a critical bionetwork for its bioactive residents which includes growth factors.

Matrix as Growth Factor Modulator

Substantial evidence is accumulating that structural proteins can bind to growth factors and regulate their

activities. A good example of this paradigm comes from in depth biochemical, cell, and genetic analysis of the heparan sulfate proteoglycans (HSPGs). Though not technically "matrix" proteins, they may interface with matrix and ultimately affect the bioavailability of growth factors in ways described below. HSPGs reside either intercalated in the cell membrane (syndecans) or tethered at the cell surface (glipicans) (for review see [7]). They directly bind grown factors including FGF-1 and 2 [8], gamma interferon, interleukin 8, PDGF-AA, platelet factor 4, and the BMP antagonist noggin [9]. It is believed that the HSPGs orient and position the growth factors close enough to their receptors so that they become activated. Another working paradigm is that the matrix is a "holding facility" or a storage bank for growth factors [10]. HSPGs are abundant in bone tissue [11] and expressed by hematopoetic and nonhematopoetic bone marrow stroma cells [12].

The importance of the HSPG perlecan to bone is evident in both spontaneous and genetically engineered mutations that cause severe lethal skeletal abnormalities [13, 14]. The removal of the polysaccharide containing proteoglycans is equally as important, as indicated by the human condition mucopolysaccharidosis type VI (MPS VI). A feline counterpart with this mutation acquires osteopenia because of defects in bone formation [15], highlighting the concept that proper PG turnover is critical to normal skeletal function. Some HSPGs are cleaved into fragments that assume roles beyond the cell surface. One prominent HSPG in this category is called "endostatin" and is derived from carboxyterminus of the modular type XVIII collagen. It has received considerable attention in recent years because of its powerful (but somewhat inconsistent) inhibition of angiogenesis and tumor formation *in vivo*. Although we cannot be certain what role HSPGs play in bone cell function, it is likely that mechanisms involving growth factors such as VEGF or FGF will be involved [16] and that the concept of "matrix and modulator" will hold true in mineralized tissues.

It is further tempting to speculate that a fine equilibrium must be attained to "tame" growth factors so they perform properly. Considering new estimations that the human genome may encode a mere 40,000 genes, the fact that growth factors could interact with either the core proteins or the heterogeneous glycosaminoglycan chains (GAGs) could provide additional intricacy of function. Thus, it is conceivable that numerous combinations of growth factor interactions can activate or repress skeletal cell functions, depending on the needs of the organism. This conceptual complexity could hold true for many of the other matrix proteins that are highly posttranslationally modified and, in some cases, have alternatively spliced mRNA and protein variants.

Another class of bone matrix proteins that binds growth factors is the small leucine-rich proteoglycans (SLRPs). They bind TGF-beta and to other matrix proteins including collagen, fibronectin, and thrombospondin [6]. The potential relationship of SLRPs to TGF-beta function in bone is beginning to emerge using skeletal cells from KO mice deficient in the SLRP called biglycan. Cultured bone marrow stroma cells and more mature calvarial cells respond poorly to TGF beta [17] and BMP-2, respectively (X-D. Chen and M. Young, unpublished results) leading one to predict that this SLRP could act as a "traffic monitor" directing growth factors to their receptors. When all sites are occupied, factors can either be re-directed or stored for further use. Localization studies corroborate this working theory: biglycan is found localized pericellularly in both bone and cartilage tissues [18]. Further complexity of function could arise from additional interactions with other TGF beta-binding proteins such as LTBP or other SLRPs such as decorin or fibromodulin. Clearly, much more work is needed to unravel the precise sequence of events that control powerful cytokines such as TGF beta and its related family members the BMPs.

Signaling: Not Just for Growth Factors

Several lines of evidence using purified matrix proteins or mice genetically engineered to over- or under-produce all or parts of them show that they have key roles in many cellular functions. Early *in vitro* data using collagen-treated osteoblast cells showed modulation of cell activities including cell shape, proliferation [19], differentiation [20], and mineralization [21]. Some *in vivo* examples illustrating this point come from "knockin" mice with site-directed mutation of the collagenase cleavage site: they have increased apoptosis in osteocytes and increased bone formation [22]. Concepts on how specific matrix proteins may be affecting bone cell function come from several papers by Taleuchi and Matsumoto [23], the most recent of which shows that collagen-elicited FAK and ERK signaling converges with BMP signaling at the level of SMAD activation in the nucleus. Studies like these help matrix function transcend from a simple scaffold model to one outlining direct influences on the complicated signaling events that control osteoblast differentiation.

Another category of proteins residing in the mineralized matrix with signaling functions are called the SIBLINGS (Small Integrin-Binding LIgand, N-linked Glycoprotein) [24]. The integrin-binding component of the family name refers to the fact that they all have RGD cell attachment sequences that can (but not always) assist in integrin receptor binding. Genes encoding the family are clustered in tandem on human chromosome 4 and include osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP 1) dentin sialophosphoprotein (DSPP), and matrix extracellular protein (MEPE). One member of the family,

osteopontin, has even been referred to as a "cytokine" [25] and appears to have numerous direct functions on cells affecting immunity, metastasis, "mechano-sensing," hormone action, and bone resorption. The proposed functions of the SIBLINGS as well as other bone cell attachment matrix proteins such as fibronectin, thrombospondin, osteonectin, and vitronectin are so numerous they cannot be listed here and are reviewed in [6]. The importance of matrix proteins like matrix gla (MGP) in controlling mineralization is evident coming from both biochemical and genetic lines of experimentation (reviewed in [6]). Diminished production of MGP either by warfarin treatment (which inhibits the gamma carboxylation of the protein) or by gene knockout in mice results in massive ectopic calcification, leading to the conclusion that it is a potent inhibitor of mineralization. Taken together, one can conclude that a fine balance of matrix protein expression must be maintained and that the matrix itself has direct roles in calcified tissue structure and function.

Final Commentary

One could argue that matrix proteins are like an American "fast food" meal: a lot of bulk but very bland. My counterpart may argue: if the mineralized matrix is so important why is it so abundant? It's true, with growth factors a little goes a long way. But to be fair, neither matrix nor growth factors could exist alone and should be considered as a "functional unit" [26] like café-au-lait. Growth factors have power that needs to be harnessed; matrix proteins provide "cues" or direction so that growth factors can provide sustained, regulated functions within calcified tissue.

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